JOURNAL OF AGRICULTURAL RESEARCH

VOL. 70 WASHINGTON, D. C., MAY 1, 1945 NO. 9

A METHOD FOR DETERMINING THE RELATIVE COLD HARDINESS OF DORMANT PEACH FRUIT BUDS[1]

By E. M. MEADER, formerly *junior pomologist*[2]; O. W. DAVIDSON, *assistant professor of pomology;* and M. A. BLAKE, *chief in horticulture, New Jersey Agricultural Experiment Station*

INTRODUCTION

In the more northern States where peaches are grown commercially, the fruit buds are frequently damaged by winter weather. In 3 years of the past decade there has been considerable injury to peach buds in commercial orchards in New Jersey, especially to those of the relatively tender varieties. Exceptionally cold winters are sometimes referred to by pomologists and plant breeders as "test" winters, since the relative hardiness of different varieties of fruit can be determined by observing the amount of injury sustained by each as a result of the low temperatures.

One of the limitations to an extensive breeding program to originate hardier peach varieties for northern districts has been the inability to evaluate the cold hardiness of seedlings and varieties without their experiencing a "test" winter. The evaluation of fruit-bud hardiness for a variety of peach is a long and uncertain task, as measured by uncontrollable outdoor conditions. Trees of a variety to be tested must be propagated, grown to fruiting age, and then subjected to critical low temperatures before hardiness determination is possible. A reliable artificial method for testing the cold hardiness of peach fruit buds should therefore be of great value to peach breeders. By the use of such a method trees of newly introduced varieties successful in other peach districts and promising seedlings from the breeding work might be rated for hardiness as grown under orchard conditions in New Jersey and elsewhere before any "test" winter occurs. A practical freezing test for determining the relative cold hardiness of the fruit buds of different varieties of peaches is described herein.

REVIEW OF LITERATURE

Many investigators have studied winter hardiness in plants, and numerous methods have been proposed for measuring the resistance

[1] Received for publication July 26, 1943. This paper was submitted in thesis form by the senior author to the Graduate School of Rutgers University in partial fulfillment of the requirements for the degree of master of science. It presents the results of cooperative investigations of the New Jersey Agricultural Experiment Station and the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. Journal Series paper of the New Jersey Agricultural Experiment Station, Division of Horticulture.

[2] Now assistant pomologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

of plants to cold. Excellent bibliographies, including many references to orchard observations on the hardiness of fruit trees, have been compiled by Chandler (*8*),[3] Hildreth (*17*), and Potter (*27*). For convenience the methods have been grouped as follows: (1) Indirect determinations that may be correlated with hardiness; (2) direct determinations wherein plants or plant parts are frozen under controlled conditions.

INDIRECT DETERMINATIONS

Beach and Allen (*3*) investigated structure and composition as related to hardiness in the apple. Johnston (*19*) and Strausbaugh (*32*) reported that the moisture content of the buds was directly related to hardiness in the peach and plum. Bakke et al. (*2*), Dunn and Bakke (*15*), and Dunn (*14*) studied relationships of freezing-point lowering of the cell sap, moisture content, ash determination, and a dye adsorption test to hardiness in the apple.

To rate hardiness in alfalfa and in apple, Dexter (*11*), Swingle (*34*), and Stuart (*33*) made use of electrical conductivity measurements of the diffused electrolytes from tissues frozen artificially. Stark (*30*, *31*) reported that a hardy apple variety retained more unfrozen water at low temperatures than did a tender variety. Tysdal (*35*) developed a method for studying the relationship of varietal cold hardiness in alfalfa to enzymatic activity. Levitt and Scarth (*20*, *21*), in studies on woody plants, used a plasmolytic method for determining osmotic pressures in living cells and suggested the use of a permeability test for predicting cold hardiness.

DIRECT DETERMINATIONS

Chandler (*8*), Allen (*1*), Mix (*23*), Carrick (*7*), Howard (*18*), and Potter (*25*, *26*), using an ice-salt mixture to cool insulated chambers to the desired low temperatures, found greater injury to woody tissues (apple, peach, and cherry) by rapid temperature fall than by slow freezing. Hildreth (*17*) designed an apparatus cooled by coils operated by a mechanical ammonia compressor and found that apple twigs exposed to −20° for 12 hours were more severely injured than those held at the same temperature for 3 hours. Magness (*22*) paraffined the cut ends of pieces of roots, placed the roots in glycerin and water, and froze them by gradually lowering the temperature of the solution to certain low points. Dunn (*13*, *14*) sealed apple twigs in glass tubes, placed the tubes in an ether bath, and froze the twigs by dropping pieces of solid carbon dioxide in the ether a little at a time. Peltier and Tysdal (*24*), Brierley and Angelo (*6*), and Hansing et al. (*16*) tested the hardiness of alfalfa, grape, and perennial sedums, respectively, by growing plants in containers that could be moved into controlled low-temperature chambers. Treated plants were put in the greenhouse to observe recovery. Cullinan and Weinberger (*10*) exposed peach fruit buds in a chamber set for the desired minimum temperatures, but experienced difficulty in getting their material to the same low point at an unaltered rate of fall on successive dates. Their results indicated that Elberta increased in hardiness during

[3] Italic numbers in parentheses refer to Literature Cited, p. 300.

November, changed little during December, and reached its maximum cold resistance on January 21, 1933, at Beltsville, Md.; Greensboro and Carman were consistently hardier than Elberta. They concluded that varietal differences in hardiness, seasonal growing conditions, size of crop, the stage of development of buds in early and late winter, and quantitative and qualitative differences in shoot growth were factors affecting the survival of buds during periods of critically low temperatures. Smith and Potter (*28*) described an improved freezing chamber for automatically controlling temperatures. Winklepleck and McClintock (*36*) rated the amount of injury to *Prunus* stocks that were exposed to $-15° \pm 1°$ F. in an insulated box for various time intervals, but no consideration of the rate of fall to the minimum temperature was given.

CHOICE OF METHOD FOR HARDINESS DETERMINATIONS

Of the several methods used by other workers, the diffusion of electrolytes method seemed to offer the greatest promise for rating hardiness of the peach, since it had been so favorably reported for other kinds of plants. A comparison of data obtained by this method in the present investigations with the published results of Blake (*5*) on hardiness of peach varieties under orchard conditions showed no consistent relation between the results of the two methods of evaluating hardiness. Besides the amount of labor and the time involved in making determinations, a number of uncontrollable factors were found to influence adversely the results by this method.

The objections associated with hardiness tests on peach by means of diffused electrolytes led the authors to seek a rapid direct freezing method for evaluating the cold resistance of peach buds. Preliminary work on this problem was undertaken in March 1940, and a simple, practical method was evolved. The description and use of this method, together with the establishment of its limitations, form the basis of this publication.

An electrically operated ice cream cabinet-type refrigerator (fig. 1) was adapted by means of simple equipment to simulate winter conditions as they occur in nature. The refrigerator was equipped with a thermostat that permitted it to operate at temperatures as low as $-30°$ F. To make possible precise settings of this thermostat, a 12-inch pointer of strip metal was soldered to the dial knob (fig. 1). The end of the pointer, when the knob was turned, followed a semicircular graduated scale inscribed on the cabinet, and produced amplificative readings of the small dial scale. The cabinet had a single compartment 21⅛ by 10⅜ by 14⅞ inches deep with a removable hinged lid that fitted tightly upon a rubber insulating collar at the top edges of the compartment.

A rectangular galvanized sheet-iron tank 16⅞ by 9⅛ by 12¾ inches deep, having 2 wire frames forming 4 divisions for holding test tubes and a central one for a vacuum-operated agitator (fig. 2), was placed within the compartment and allowed to rest on five 1-inch wooden blocks to prevent direct contact with the bottom of the cooling compartment. A bath containing 19 quarts of 50-percent ethanol was used in the tank. Twelve-inch glass tubes with a diameter of 1¼ inches were weighted with No. 8 lead shot so that they floated upright in the

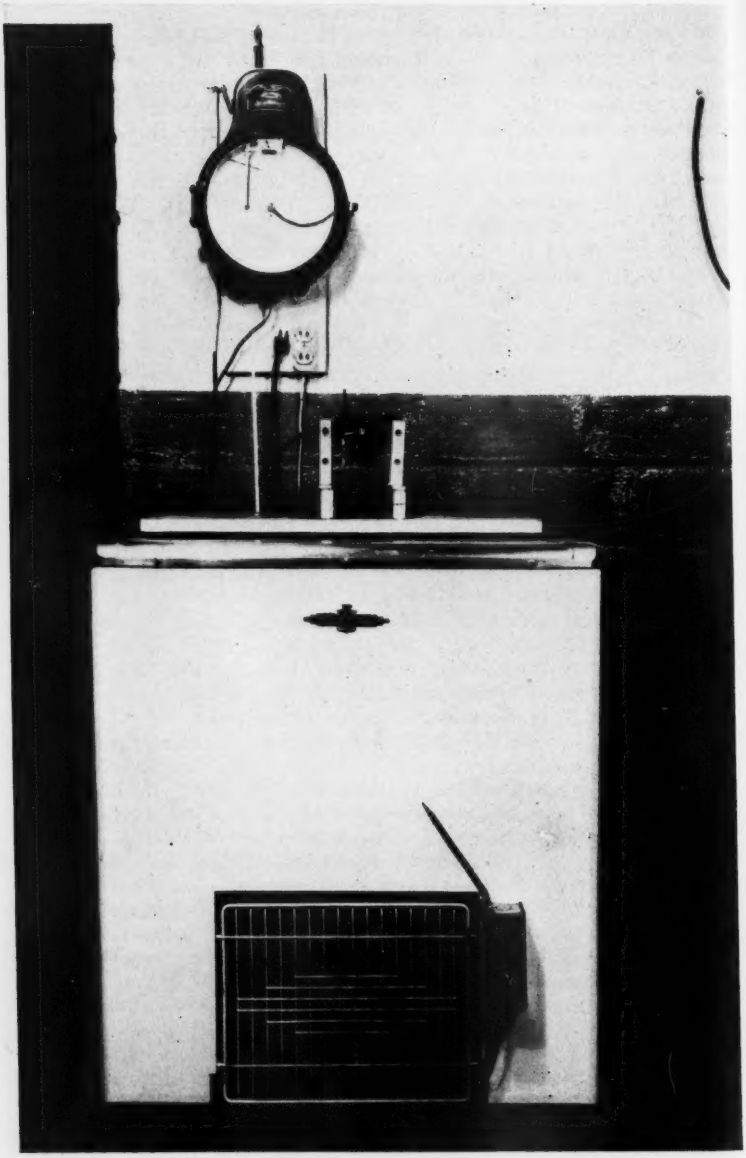

FIGURE 1.—Apparatus used for direct controlled freezing of peach buds.

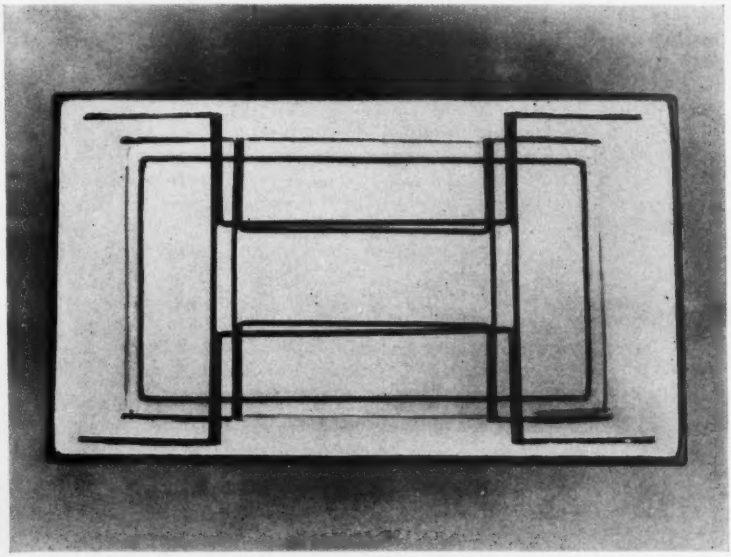

FIGURE 2.—Top view of tank, showing arrangement of compartments in which test tubes containing samples were supported in an alcohol-water bath.

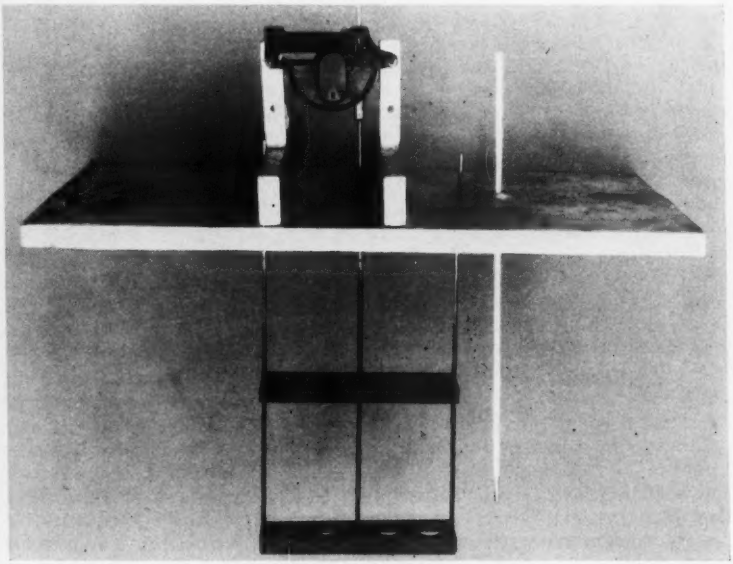

FIGURE 3.—Side view of the agitator that operated in the central compartment of the tank.

bath, which came above the bottom of the rubber stoppers used for sealing samples of twigs in the tubes. Eleven pounds of shot were used in the 50 tubes placed in the tank at one time.

A windshield wiper run by an aspirator pump attached to a nearby water faucet was used to operate the agitator (fig. 3), an arrangement which permitted the maintenance of even temperatures throughout the tank while the bath was cooling to subzero temperatures. The windshield wiper stirrer was mounted on a piece of well-painted $\frac{7}{8}$-inch white pine of the proper size to cover the compartment. The agitator operated in a vertical direction (fig. 3) and consisted of two sheet-iron blades, $1\frac{7}{8}$ by $7\frac{1}{2}$ inches, mounted one above the other and each provided with six $\frac{3}{4}$-inch holes.

An accurate thermometer graduated to $-32°$ F. and having a 16-inch stem below this graduation was inserted into the bath through a cork in the cover and adjusted to keep the bulb spaced 2 inches from the bottom of the tank. The bath temperature could be read to 1° and estimated to the nearest 0.1° without removing the thermometer or stopping the agitator.

A continuous record of the temperature changes that occurred in the bath was made by a recording thermometer with a 24-hour chart graded in degrees Fahrenheit (fig. 1, top). Since the remote extension bulb of the recording thermometer was also placed in the bath, the respective readings of the two thermometers could be checked at any time. Any differences were usually within 1° F.

PRINCIPLE FOR AUTOMATIC CONTROL OF RATE OF TEMPERATURE FALL

The antifreeze solution in the tank cooled more slowly than the air in the compartment, and this lag in the temperature change of the antifreeze gave the desired slow rate of cooling. This lag is illustrated in figure 4. Factors that influence the rate of temperature fall of the bath from a given starting temperature and with a given amount of plant material to be frozen are: The specific gravity of the antifreeze solution, its volume, the rate at which the bath is stirred, and the thermostat setting that controls to $\pm 1°$ F. the minimum temperature of the air in the compartment. In daily runs, when 50 samples were being frozen at a time, the thermostat remained unchanged. Care was taken to put the samples in at the same starting temperature and to maintain the antifreeze at the same specific gravity and volume. When this was done the rate of temperature fall could be duplicated at will, and the total time required to attain the desired low point agreed within 15 minutes for successive runs. As soon as the desired minimum temperature was reached, samples were removed or the cover raised to prevent the bath from dropping to a lower temperature.

For the most part, samples were thawed by removing them directly from the tank to room temperature as soon as the minimum temperature had been reached. In an experiment to test the effects of rapid and slow thawing, the latter was accomplished by raising the cover with 2-inch wooden blocks, one under each end, and forcing air at room temperature ($70°-75°$ F.) into the bath compartment by means of an electric fan while the agitator still operated. This procedure could also be used to raise the bath to the desired starting temperature, but

it was found more satisfactory to remove the tank and place it on a steam radiator in the room because when this was done there was less evaporation of alcohol, and a shorter time was required to reach temperatures above 32°. Samples were allowed to thaw in the sealed tubes until all frost on the twigs had disappeared.

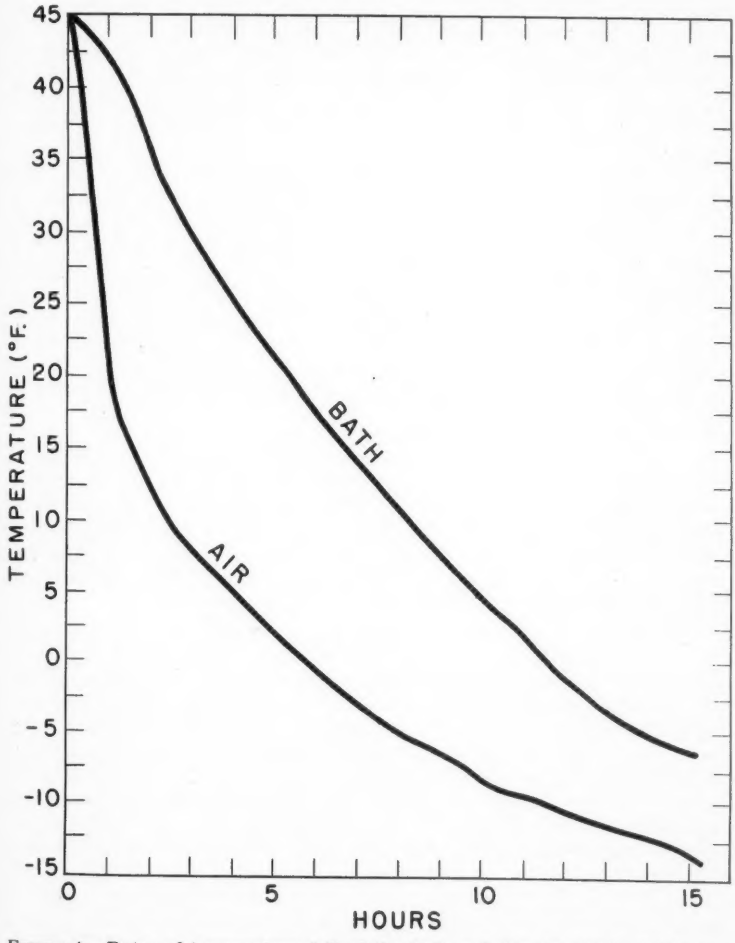

FIGURE 4.—Rates of temperature fall of the bath and the air within the freezer cabinet.

After thawing, twigs were removed from the tubes and allowed to stand in pans of water for at least 24 hours before the buds were examined for injury. After this time interval the fruit buds were cross-sectioned with a razor blade. Injured buds were characterized by a marked brown discoloration, and a water-soaked appearance.

PREPARATION OF SAMPLES

In preliminary studies in 1940 and 1941, samples of the varieties listed in table 1 were taken from orchards of the New Jersey Agricultural Experiment Station at New Brunswick. Uninjured and unbranched terminal twigs 12±1 inch in length and with an optimum number (average number or more for a given variety per 12-inch twig) of buds per twig were selected from the periphery of a tree. The bud set was determined according to the New Jersey standard (*4*). The twigs were cut at either top or bottom to 10½ inches in length, with loss of as few buds as possible. Ten to 15 twigs were then carefully nested together in each glass tube, and the latter sealed with a rubber stopper.

Beginning with experiment 3, all samples were taken from the Adams orchard in Franklin Park, about 8 miles from New Brunswick. Large composite samples of buds were gathered from at least 10 trees of each of the varieties that were used in these studies. The twigs were brought immediately into the laboratory, cut to length, randomized into samples of 10 or 12 twigs, and sealed in tubes ready for freezing.[4] Both 12 ±1-inch and 18 ±1-inch twigs were used. The longer twigs were cut at their midpoints to accommodate them to the tubes. Comparable samples were then given certain treatments as noted under each experiment, a description of which follows.

PRESENTATION OF DATA

Several preliminary trials were made to learn the proper thermostat settings to give a desired rate of temperature fall to a particular minimum temperature. Eight peach varieties known to have wide differences in the hardiness of their fruit buds were used to ascertain the rate of cooling to subzero temperatures that would most effectively differentiate the hardiness of these varieties.

EXPERIMENT 1

On March 28, 1940, the starting temperature of the bath was 37° F., and −8.5° was reached in 19 hours. The starting temperature on March 29 was 39°, and the minimum of −7° was reached at the end of 28 hours. A comparison of data for this experiment with those obtained by Blake (*5*) at New Brunswick, N. J., for the same varieties under orchard conditions following a temperature of −3° on February 13, 1933, is given in table 1. The mean diameters of twigs in hundredths of an inch and their bud set per 12 inches for the varietal samples are also shown.

[4] Twigs placed in water for several hours either in the laboratory or in cold storage developed more bud injury during the freezing tests than comparable twigs not placed in water.

TABLE 1.—*Data obtained in artificial freezing of dormant peach buds compared with data for buds frozen under orchard conditions; experiment 1*

Variety	Mean diameter (hundredths of an inch) of twigs frozen artificially					Frozen under orchard conditions Feb. 13, 1933 [1]	
	Top	Bottom	Bud set per foot	Total number buds	Percent buds alive	Bud set per foot	Percent alive
	Mar. 28, 1940						
41 S. D.[2]	11.8	15.4	25	182	56	18	65
Dewey	10.3	14.0	15	113	51	12	85
Golden Jubilee	9.9	14.4	22	158	37	18	38
Triogem	10.3	15.1	19	134	28	20	13
Cumberland	10.9	15.1	28	141	21	32	23
Slappey	10.8	14.2	15	138	20	17	30
White Hale	10.8	14.8	13	101	5	16	7
Elberta	10.9	14.6	12	94	3	13	5
	Mar. 29, 1940						
41 S. D.	13.8	17.1	25	145	74	18	65
Dewey	12.8	17.0	15	135	61	12	85
Cumberland	11.7	15.6	28	123	41	32	23
Slappey	11.6	15.5	15	164	41	17	30
Golden Jubilee	10.9	15.2	22	149	38	18	38
Triogem (N. J. 70)	11.4	15.6	19	109	21	20	13
Elberta	12.7	16.8	12	132	5	13	5

[1] These data were reported by Blake (*5*) for samples of 100 buds taken from orchard-growing trees that were subjected to a temperature of −3° F. on Feb. 13, 1933.
[2] Selected seedling from cross of Slappey×Dewey.

EXPERIMENT 2

Freezing tests were resumed in the winter of 1940–41, and on January 2, 50 varieties were frozen from a starting temperature of 42° to −4° F. in 15 hours. On January 3 the starting temperature was 41°, and −4° was reached in 17 hours. The thermostat setting was unchanged, but some water was added to the bath to give the proper volume. This caused a 2-hour delay in freezing, a fact that shows the necessity for checking the specific gravity of the solution if a duplicated rate of freeze is desired. A few of the 50 varieties frozen that have a wide range in relative hardiness are listed in table 2 for comparison with orchard data.

The following experiments designed to ascertain the effects of (1) various rates of freezing; (2) different starting temperatures of the bath; and (3) various critical low points for removal of samples when the rate of fall had been the same, were performed to establish the limitations of technique for the direct freezing method.

The number of live and killed buds on each of the 12- and 18-inch twigs was recorded. Variation in the percentage of live buds on different twigs occurred. Chi-square values were determined according to methods of Snedecor (*29, pp. 164–167*) to see if these variations were within the limits of random sampling from homogeneous material. The data for several twigs were grouped at random for com-

puting chi square in R × 2 tables. Each group contained at least 50 to 100 buds. When only sampling variation from the mean percentage of buds alive for a variety resulting from a given treatment had been proved, differences in the percentages of buds alive in the different treatments were tested by chi square calculated in 2 × 2 tables with a single degree of freedom. Significant differences between treatments are indicated in the tables that follow.

TABLE 2.—*Data obtained in artificial freezing experiments of dormant peach buds compared with data for natural freezing injury in orchard; experiment 2*

Variety	Frozen artificially				Frozen under orchard conditions [2]
	Jan. 2, 1941		Jan. 3, 1941		Feb. 13, 1933
	Total buds	Buds alive	Total buds	Buds alive	Buds alive
	Number	*Percent*	*Number*	*Percent*	*Percent*
Marquette No. 1 [1]	174	77.5	193	62.6	
Marquette No. 2			178	68.5	
41 S. D. No. 1	148	64.2			65
41 S. D. No. 2			175	51.4	
Duke of York			122	55.7	54
Early Rose	239	60.7	188	51.1	
Greensboro	246	53.3	175	46.6	90
Veteran	105	54.3			
Golden Jubilee			180	32.2	38
Garden State [3]	107	21.5	123	17.9	
Cumberland	249	20.9	246	22.0	23
Hiley	216	19.9	181	9.4	5
Elberta			98	14.3	5

[1] Trees 1 and 2 sampled.
[2] Data by Blake (*5*); 100 selected buds of each variety were examined.
[3] Nectarine.

EXPERIMENT 3

The effects of rate of temperature fall are closely associated with the starting temperature, the following treatments being used: (1) Samples put in bath at 38° F. and cooled to −6° in 13½ hours; (2) Samples put in bath when it had dropped to 20° and cooled to −6° in 9½ hours; (3) samples put in at 10° and lowered to −6° in 6½ hours; and (4) samples put in at 0° and reached the minimum of −6° in 3 hours. Samples in treatments 2, 3, and 4 were kept at 45° until put in the bath when this had reached the temperatures indicated. All samples of the four treatments reached the minimum of −6° simultaneously and were thawed similarly, a return to 39° being accomplished in 6 hours; that is, the treatments varied only in the time and temperature at which they started. To maintain the level of the bath just above the bottom of the stoppers in the tubes and yet not change its volume when tubes of different treatments were removed from or put into the tank, bottles weighted with dry sand were used to displace the solution in amounts equal to the volume of the tubes (usually 12 per treatment) included in the treatments. The thermometer was carefully watched to see when the bath started to cool again after the samples of each treatment (at approximately 45°) were added. This occurred in less than one-half hour in each case. Data obtained in experiment 3 are presented in table 3.

TABLE 3.—*Data on effects of rate of temperature fall and starting temperatures on survival of dormant peach fruit buds of 2 varieties, Feb. 3, 1941; experiment 3* [1]

Treatment			Summercrest		Cumberland	
No.	Starting temperature of bath	Time elapsed before temperature dropped to —6° F.	Total buds	Buds alive	Total buds	Buds alive
	°F.	*Hours*	*Number*	*Percent*	*Number*	*Percent*
1	38	13½	344	61.6	405	53.1
2	20	0½	378	50.0	428	35.3
3	10	6½	410	2.2	457	2.8
4	0	3	366	0	452	0

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparison was as much as 8.5, and less than 1 percent where percentage difference in treatment comparison was as much as 11.6.

EXPERIMENT 4

The purpose of experiment 4 was to determine whether measurable differences in injury to fruit buds would occur if buds were taken from the cool temperatures of the orchard to a warm room and, after being prepared there, frozen to the desired low points. If differences did occur, the alternative would be to prepare the samples at the temperature prevailing in the orchard and then have the bath at the same temperature before putting in the samples. The effect of putting in samples at 20° and 10° F. for starting temperatures of the bath and freezing them to –6°, but at a slower rate than in experiment 3, was also tested.

TABLE 4.—*Data on the effect of certain starting temperatures and a relatively slow rate of freeze on survival of dormant fruit buds of 4 varieties of peaches, Feb. 10, 1941; experiment 4* [1]

Treatment				Summercrest		Cumberland		Triogem		Golden Jubilee	
No.	Initial temperature of buds	Starting temperature of bath	Time elapsed before temperature dropped to —6° F.	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	°F.	°F.	*Hours*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	45	40	21¾	439	75.4	767	76.3	512	78.9	819	85.2
2	83	40	21¾	496	81.7	661	72.0	497	83.1	614	85.8
3	45	20	14¼	455	63.1	609	50.9	530	68.9	715	47.1
4	45	10	8¾	509	7.5	674	7.0	515	8.2	661	2.6

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparisons was as much as 6.3; and less than 1 percent where percentage difference in treatment comparisons was as much as 14.2.

After being sealed in the tubes, all samples were kept at 45° F. until they were treated as follows: (1) put in the bath with a starting temperature of 40° F. and frozen to –6° in 21¾ hours total time; (2)

samples brought to 83°, as determined by a thermometer inserted through a stopper into the center of the twigs in one tube, and then put in the bath simultaneously with treatment 1; (3) samples put in the bath when it had dropped to 20° and lowered to −6° in 14¼ hours; (4) samples put in the bath at 10° and lowered to −6° in 8¾ hours. When the minimum of −6° was reached, the temperature of the bath was brought up to 28° before all samples were removed to room temperature. Relevant data are given in table 4.

EXPERIMENT 5

To ascertain the most practical starting temperature of the bath, samples of the same four varieties that were used in experiment 4 were prepared and kept at 45° F. until given the treatments indicated in table 5.

These treatments, as in the previous experiments, provided for successively greater and faster drops from the initial temperature (45°) to the bath starting temperature with decreasing time intervals to the minimum temperature of −6°. All samples remained in the bath until it had returned to 36° in 3¼ hours. The summarized data appear in table 5. For the first time after chi square tests for homogeneity of buds on the pooled 12-inch and 18-inch twigs had been made, it was found necessary to separate the two lengths in order to have uniformity within samples of buds that had been treated similarly throughout the experiment. This applied to the Cumberland and Golden Jubilee varieties, which apparently had consistently hardier buds on 18-inch twigs than on 12-inch twigs, but not to Summercrest or to Triogem.

TABLE 5.—*Data on effect of bath starting temperatures as related to survival of peach fruit buds of 4 varieties and the buds on 12-inch and 18-inch twigs when frozen to a minimum of −6° F., Feb. 13, 1941; experiment 5* [1]

Treatment			Summercrest		Cumberland				Triogem		Golden Jubilee			
					12-inch twigs		18-inch twigs				12-inch twigs		18-inch twigs	
No.	Starting temperature of bath	Time elapsed before temperature dropped to −6° F.	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
		Hours	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	40	15½	356	62.1	566	48.6	380	52.1	414	66.2	257	49.4	149	68.5
2	30	12¾	321	60.4	269	30.1	204	41.2	402	55.2	239	47.3	199	57.8
3	27.5	12	359	59.3	296	31.4	221	47.5	344	57.0	254	49.2	171	54.4
4	25	11½	310	68.1	343	24.8	221	41.2	349	61.0	229	31.9	217	54.8
5	22.5	10¾	333	45.9	286	17.1	194	42.3	332	53.3	246	26.4	188	39.4
6	20	9¾	311	46.3	283	13.4	184	29.9	383	41.0	239	19.3	222	41.9

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparisons was as much as 7.7; and less than 1 percent where percentage difference in treatment comparisons was as much as 10.9.

EXPERIMENT 6

The effect on peach buds of a difference of 1° or 2° within the critical range of minimum temperatures at which buds are injured, when the

rate of fall from the same bath starting temperature was similar for all samples, was determined, as shown in table 6. N. J. 66, which is known to have tender fruit buds easily injured by subzero temperatures under orchard conditions, was substituted for Triogem in this and the following experiment. Twelve tubes of each of the four varieties tested were placed in the bath at 30°. Since the tubes had been held at room temperature (70°–75°), they raised the bath to 38° before it started to cool. When subzero temperatures were reached samples were removed from the bath at six low points as shown in table 6. The time interval for the bath to reach each minimum temperature from its starting temperature of 38° is also listed. All samples, after removal from the bath, were allowed to thaw at room temperature in the sealed tubes.

TABLE 6.—*Summary of data on the effect of 6 subzero temperatures on the survival of fruit buds of 4 peach varieties frozen simultaneously at the same rate of temperature fall, Feb. 25, 1941; experiment 6* [1]

Treatment			Summercrest		Cumberland		N. J. 66		Golden Jubilee	
No.	Minimum temperature	Time elapsed before temperature dropped to low point	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	°F.	*Hours*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	−4	13¾	321	82.2	464	71.6	310	41.3	387	86.8
2	−6	15	340	71.5	468	57.9	357	[2] 41.2	347	70.6
3	−7	16¼	330	60.3	498	38.2	317	17.7	380	58.2
4	−7.5	17¼	373	53.6	502	30.7	347	[2] 19.9	369	56.1
5	−8	18	309	46.0	470	30.4	386	16.2	367	49.9
6	−9	19¼	298	37.6	499	21.0	318	16.0	352	41.8

[1] *P* value for chi square less than 5 percent where percentage difference in treatment comparison was as much as 7.5; and less than 1 percent where percentage difference in treatment comparison was as much as 9.4.
[2] *P* value in chi square test for homogeneity less than 5 percent.

EXPERIMENT 7

Experiment 7 was similar in purpose and details to experiment 6, except that the rate of freezing to lower temperatures was relatively much slower. Five progressively lower settings of the thermostat were made to obtain prolonged slow cooling (an average change of approximately 1.25° per hour) to subzero temperatures from the bath starting temperature of 32° F. The six treatments and the data obtained are shown in table 7.

DISCUSSION OF FREEZING METHOD AND DATA OBTAINED BY ITS USE

APPARATUS

No record was found in the literature of the direct use of the lag of a bath of antifreeze solution behind the air temperatures in a cooling chamber for automatically producing the desired rates of temperature fall to subzero temperatures. The apparatus described herein is simple in construction, requires no extra insulation, and with reasonable care

in operation will give continued good service. An important advantage to controlling the temperature of a bath over attempting to maintain air temperatures in a chamber at desired points is that samples may be put into the bath at different times or removed at subzero temperatures without causing appreciable fluctuations in its temperature.

TABLE 7.—*Summary of data on the effect of subzero temperatures on survival of peach fruit buds of 4 varieties frozen simultaneously at the same prolonged slow rate of temperature fall; experiment 7, Feb. 27, 1941* [1]

Treatment			Summercrest		Cumberland		N. J. 66		Golden Jubilee	
No.	Minimum temperature	Time elapsed before temperature dropped to low point	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive	Total buds	Buds alive
	° *F.*	*Hours*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	−6	30¼	306	51.6	551	[2] 64.1	370	66.5	368	85.3
2	−8	31¼	362	37.8	550	[2] 52.5	344	48.5	380	80.5
3	−9	32¾	400	27.8	678	35.1	365	38.4	391	64.7
4	−11	34	360	12.8	449	18.0	354	11.0	370	55.9
5	−13	35¾	308	.6	491	4.5	376	1.1	386	7.0
6	−15	37¾	385	0	488	.4	353	.6	359	1.1

[1] *P* value for chi square less than 1 percent where percentage difference between treatments was as much as 8.8.

[2] *P* value in chi square tests for homogeneity less than 1 percent.

It is essential that the antifreeze solution used should give adequate protection at the low temperatures that the operator of the apparatus desires. If ice crystals start to form in the bath, the drop in temperature is retarded for a time because of the liberation of latent heat, and the agitator is run with difficulty. An antifreeze solution of ethyl alcohol and water was chosen because the mixture was readily available and relatively inexpensive, and it had the decided advantage of leaving the tubes clean and dry for handling. Evaporation made necessary the occasional replenishing of the bath with alcohol to give the desired specific gravity for freezing protection at subzero temperatures and to maintain its original volume. A hydrometer of the type commonly used for testing solutions in automobile radiators was found convenient for testing the freezing protection of the bath. To equalize temperatures throughout the tank, the bath was thoroughly stirred at all times while cooling.

DATA TABLES 1 AND 2

From the data presented in tables 1 and 2, it can be seen that when Elberta is so frozen artificially that nearly all its buds are killed (1 to 15 percent alive), the other varieties listed compare favorably with orchard data by Blake (*5*) for the same varieties at New Brunswick. The differences observed may be due to different degrees of hardiness of trees within the same variety or to the vagaries of sampling. Beginning in January 1941, this method for hardiness determinations of peach fruit buds by direct controlled freezing was put into daily practical use. Fifty samples were prepared and frozen in such a

manner as to leave less than 15 percent of the buds of Elberta alive for comparative purposes. Some hardy varieties, like Marquette, or 41 S. D., were also included in each lot of samples. With these varieties for criteria, the fruit-bud hardiness of varieties or seedlings the hardiness of which had not been tested in cold winters or was unknown could be evaluated. In the case of crosses, samples of parental varieties were frozen along with samples of their progeny. It is necessary to anticipate the type of freeze (rate of temperature fall to a certain low point for removal of samples) that will permit between 1 and 15 percent of the buds of a tender variety like Elberta to remain alive. When this is done, individuals more tender than Elberta can be recognized. If a procedure similar to those of experiments 6 and 7 is used, the proper subzero temperature for removal of samples to give the desired injury to Elberta buds can be determined in one trial freeze. In early winter it may be necessary to use progressively lower minimum temperatures before removal of samples when a particular rate of temperature fall is used, since there is a general increase in the hardiness of peach fruit buds during this period (*10*).

DATA TABLES 3 AND 4

When the rate of cooling from the starting temperature of 45° F. to the same subzero temperature (−6°) increases, the injury to the fruit buds also increases. This fact applies for the two freezes on the two successive dates, the second being relatively slower than the first. In parallel treatments, except for the rate of temperature fall, considerably more live buds are present in the slower freeze. As tested by chi square, significant differences between treatments are indicated in all cases, except treatments 1 and 2 in table 4. Evidently bringing samples to the laboratory, in which they experience temperatures above those prevailing in the orchard, has little effect on the results obtained by this method. Hildreth (*17*) practiced cooling his freezing chamber to the outside orchard temperature before putting samples into the freezer. Chandler (*8*) has shown that rate of thawing has no influence on the amount of injury to peach fruit buds. His findings were substantiated by unreported preliminary work in the present studies.

DATA TABLE 5

For all the treatments shown in table 5, the buds on 18-inch twigs of Cumberland and Golden Jubilee proved consistently hardier than those on 12-inch twigs. Chi square tests for homogeneity showed only variations within the limits of random sampling for the pooled data of 12-inch and 18-inch twigs of Summercrest and Triogem, but uniformity within treatments for Cumberland and Golden Jubilee was attained only by separating the data for the two lengths of twigs. Cullinan (*9*) and Cullinan and Weinberger (*10*) have recognized differences in the hardiness of peach fruit buds on qualitatively different twigs as indicated by length measurements. This fact, supported by data in this study, suggests that selected samples of similar length twigs of different varieties should be used when the buds are to be tested for cold hardiness.

In a comparison of treatments (table 5), Summercrest shows a significant difference between treatments 3 or 4, but the tendency is in

the opposite direction from that expected. A highly significant difference is shown between treatments 4 and 5, and also between treatments 3 and 5. Some unknown disturbing factor may have been responsible for the high value for treatment 4. Summercrest showed significant cold-hardiness differences in samples that were started at 27.5° and 22.5° F. respectively. Cumberland, for both twig lengths, Triogem, and the 18-inch twigs of Golden Jubilee showed significant differences between treatments 4 and 5, i. e., for samples started at 25° and 22.5°, respectively. The effect of starting samples at the several points between 40° and 20° F. was to provide a series of progressively more rapid rates of temperature fall from 45° to the particular starting temperature chosen. Chandler (*8*) froze some buds slowly one-half way down to the killing temperature and rapidly for the rest of the way, others rapidly one-half way and slowly for the rest of the way, and still others slowly all the way to the critical minimum temperature of −16° C. (3.2° F.) used in the tests. The halfway point was −12° C. (10.4° F.). He found that fast freezing in the upper part of the range (above 10.4° F.) gave the greatest injury. Elberta was the only peach variety that he used.

From the present study it is evident that rapid rates of temperature fall well above 10.4° F. can cause appreciable differences in fruit-bud injury when a critical low temperature is reached before the buds are thawed. Data from experiment 5 show the injurious effects of rapid rates of temperature fall somewhere between 30° and 20° for all of the four varieties tested. Varieties apparently react differently to the progressively rapid rates of temperature fall. It is of interest in this connection that Dorsey (*12*) has fixed 27° F. as the critical point for ice formation in peach fruit buds. That the more tender buds react first to increasing rates of temperature fall in the early stages of a freeze is well illustrated by the buds on 12-inch and 18-inch twigs of Cumberland and Golden Jubilee. The more tender buds on 12-inch twigs show a significant difference one treatment higher in table 5 than the buds on the 18-inch twigs; for example, 12-inch twigs of Golden Jubilee react significantly between treatments 3 and 4, whereas the buds on the 18-inch twigs show a significant difference between treatments 4 and 5. Thus to obtain the most efficient results with this method it seems advisable to use the same starting temperature for the bath, especially if temperatures below 35° are used. In daily runs for hardiness determinations, samples have been started between 40° and 35° to insure a gradual cooling (not over 5° to 6° change per hour) in the first part of the freeze.

DATA TABLE 6

In experiment 6 also the rate of temperature fall is the same for all samples down to the low points at which they were removed from the bath. For each degree that the temperature fell below 0° F., an increase in injury to the fruit buds occurred. No significant difference between the percentage of buds killed at —7.0° and —7.5° F., or between —7.5° and —8.0° was apparent, but for all varieties except N. J. 66 a temperature of 1° lower within the critical range of temperatures caused significantly greater injury. Of the four varieties tested in Experiment 6 N. J. 66 has the tenderest fruit buds. Under the conditions of this artificial freeze carried out on February 25,

1941, there seemed to be one level at which large percentages of the buds of N. J. 66 were killed (cf. treatments 2 and 3, table 6), but both above and below this level there were no significant changes. When examined by the chi square test for homogeneity, a tendency toward a lack of uniformity in treatments 2 and 4 was indicated.

From table 6 it appears that if there is or is not a significant difference between two varieties treated similarly (removed at the same subzero temperature), these differences or likenesses will be evident until nearly complete bud killing occurs. As the lower temperatures are reached, buds of tender varieties are more severely injured than those of hardier varieties, a factor which tends to cause greater differences between varieties in certain treatments; for example, treatments 2, 3, and 4 in comparisons of Cumberland and Golden Jubilee.

DATA TABLE 7

The weather conditions of a single winter's night are known to have caused appreciable injury to peach fruits buds in New Jersey, as well as in other peach-growing districts. If it is assumed that little change in cold hardiness of the varieties studied occurred between February 25 and 27, 1941, then the data presented in tables 6 and 7 indicate that peach buds are somewhat more resistant to cold injury during a slow rate of freezing than during the usual rate of freezing. The apparent exception in Summercrest is explained by the fact that because of lack of available 12-inch and 18-inch twigs in the labeled trees sampled on previous dates, most samples collected on February 27, 1941, were taken from other trees of this variety. The fruit buds of these trees were relatively tender on that date, as judged by the results of experiment 7.

Samples of Cumberland were also difficult to obtain for experiment 7. Lack of uniformity in the Cumberland used in treatments 1 and 2 (table 7) was indicated by chi square tests for homogeneity. Little attention would be paid to the results of these treatments, except for the fact that the data agree well with the tendencies observed for other varieties, and through all the treatments. The chi square values in the comparisons between treatments 1 and 2 for Cumberland were large for 1 degree of freedom, the P values being well beyond the 1-percent point. With the slow freezing used in experiment 7 it was possible to subject live buds of the four varieties tested to much lower temperatures than they are known to have withstood under winter conditions in the orchard at New Brunswick. Although $-5°$ F. is a critical temperature (temperature following which varieties such as Elberta usually exhibit 30 percent or more fruit-bud injury) for dormant fruit buds of most varieties of peaches at New Brunswick, buds of Golden Jubilee collected at the college farm withstood $-11°$ F. during a slow rate of artificial freezing (an average of about 1.25° cooling per hour to 0°) with relatively little more injury than was caused by $-6°$ during a moderate rate of artificial freezing (an average of about 2.5° to 2.8° cooling per hour to 0°). These results agree with orchard observations over the country. In districts north of New Jersey where maximum winter temperatures are not so high as a rule, most varieties of peaches withstand somewhat lower winter temperatures than in New Brunswick.

SUMMARY AND CONCLUSIONS REGARDING DIRECT CONTROLLED FREEZING FOR HARDINESS DETERMINATIONS

Direct controlled artificial freezing of dormant fruit buds according to methods outlined herein provides a rapid and reliable method for estimating the relative cold hardiness of fruit buds of different varieties of peach. Relatively simple equipment that can be readily manipulated to simulate natural freezing conditions was used. Slow rates of cooling to subzero temperatures (average change of 2° to 3° F. per hour) were obtained by using a constantly stirred bath of antifreeze placed within the compartment of a commercial ice-cream refrigerator. As many as 50 samples could be frozen at a time. When varietal samples were so frozen artificially that only 1 to 15 percent of the buds of Elberta, a criterion variety, remained alive, other varieties tested had percentages of live buds that compared favorably with their previous response in orchards. Variations occurred in the hardiness of buds of the same variety on qualitatively different twigs. Because of this, care in the selection of samples must be practiced. Suggestions for sampling are given herein.

The data presented emphasize the importance of the rate of temperature fall in the development of cold injury to peach fruit buds. When the rate of cooling was the same, a drop of 1° F. within the critical subzero temperature range caused a significant increase in injury to peach fruit buds of the varieties tested. Under prolonged slow rates of cooling to subzero temperatures, peach buds were found to tolerate minimum temperatures appreciably below those that the same varieties are known to have withstood under orchard conditions at New Brunswick, N. J.

When using this controlled direct-freezing method for cold-hardiness determinations, it is advisable to include more than one criterion variety in each test. The response of these criterion varieties serve as standards for estimating the relative cold hardiness of the other varieties employed in the test. This procedure is a prerequisite to the use of the direct freezing method, since the temperatures prevailing in the orchard for several days before the collection of samples, and possibly other factors as well, may exert considerable influence on the cold hardiness of peach buds during the dormant season. The results obtained also indicate the advisability of testing the cold hardiness of a variety two or more times during the dormant season.

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PHYSIOLOGIC SPECIALIZATION IN THE TOMATO WILT FUNGUS FUSARIUM OXYSPORUM F. LYCOPERSICI[1]

By L. J. Alexander, *associate plant pathologist, Ohio Agricultural Experiment Station, and* C. M. Tucker, *professor of botany, Missouri Agricultural Experiment Station*

INTRODUCTION

In 1939 Bohn and Tucker[2] described the resistance of *Lycopersicon pimpinellifolium* Mill., accession 160, to the tomato wilt fungus, *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. They[3 4] studied the inheritance of resistance in *L. esculentum* Mill. × *L. pimpinellifolium* (acc. 160) hybrids and concluded that resistance was governed by a single, dominant Mendelian factor. The senior author of the present paper, working at the Ohio Agricultural Experiment Station, crossed *L. pimpinellifolium* (acc. 160) and *L. esculentum* var. Bonny Best, but inoculation experiments failed to duplicate the results reported by Bohn and Tucker. Two fusarium isolates were used. One came from a diseased plant in a glasshouse near Cleveland, Ohio, and the other was obtained from Dr. F. L. Wellman, of the United States Department of Agriculture.

In seeking an explanation for the divergent results, cultures of the fusarium were interchanged between the writers. Subsequent investigations carried on at both experiment stations show that the conspicuously different responses to inoculation were caused by marked differences in the pathogenicity of the isolates. Bohn and Tucker[5] and Wellman and Blaisdell[6 7], as well as earlier investigators who tested the pathogenicity of isolates of the tomato wilt fungus, reported differences in the pathogenicity of the isolates studied. However, there are no reports known to the writers of the occurrence of physiologic specialization or physiologic races of *Fusarium oxysporum* f. *lycopersici* with the ability to infect and cause wilting of tomato varieties that are highly resistant to other isolates of the fungus known to be virulently pathogenic to susceptible varieties.

METHODS

The techniques used at the two experiment stations to test tomato plants for resistance to wilt were different, and, since the resulting incidence of disease differed somewhat when the same tomato lines and the same isolates of the wilt fungus were used, both methods are given in detail.

[1] Received for publication July 20, 1943. Joint contribution from the Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, and the Department of Botany, Missouri Agricultural Experiment Station. Missouri Agricultural Experiment Station Journal Series paper No. 897.

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[3] ——— and Tucker, C. M. See footnote 2.

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The technique used at the Ohio station was as follows: Seedlings were grown in sterilized soil in flats for 4 to 6 weeks. Inoculum was prepared by growing the fungus in Petri dishes on potato-dextrose agar made with 200 gm. of potatoes and 1.75 percent each of dextrose and agar per liter of medium. When cultures were ready for use, the agar with the mycelial mat was scraped into a rotary fruit press and thoroughly macerated. Sufficient water was stirred into the agar-mycelium mixture to give the inoculum the consistency of a moderately thick soup. At the time of inoculation the plants were removed from the soil in the flats and the roots were washed. The roots were then dipped in the agar-water suspension of the fungus and transplanted. As the plants wilted and died, they were marked by short wire stakes. Records for resistance were taken when no more plants died, usually about 5 weeks after inoculation. When tests for wilt resistance were being conducted an effort was made to keep the air temperature of the glasshouse at 70° F.; however, the temperature fluctuated between 60° and 85°. The soil temperature of the test bed was much more constant and was maintained at about 80° by means of a soil-heating cable and thermostat.

The technique used at the Missouri station was as follows: The seeds were planted in sterilized soil in 8-inch clay pots. The inoculum was prepared by growing the fungus on sterile oats in pint jars and was about 25 days old when used. A water suspension of the oat cultures (1 pint of oats to 1 gallon of water) was poured over the seed at the rate of 300 ml. per pot before the seed was covered with soil. Twenty-five seeds were planted in each pot. At frequent intervals plants with definite symptoms of wilt were removed and the number recorded. Final records were taken 7 weeks after planting. In some experiments the seedlings were transplanted when 1 month old and inoculated by dipping the roots in a similar oat suspension; sometimes a second transplanting and inoculation were made 1 month later. The air temperature of the glasshouse was maintained at about 80° F. with little fluctuation.

The methods of recording disease resistance were similar at both locations. "Healthy" was used to describe plants with no external symptoms of the disease or vascular discoloration in the stem when cross-sectioned near the soil level. "Vascular discoloration" was used to describe plants with no external symptoms of the disease but with vascular discoloration. "Severely diseased" was used to describe plants which died from disease or which had severe symptoms of disease during the progress of the experiment.

Four isolates of the fusarium wilt fungus were used. These are designated as Ohio 39, Washington 5, and Missouri 6 and 10. Ohio 39 was isolated by the senior author in 1939 from a diseased tomato plant found at the Berea Greenhouse Co., Berea, Ohio. Washington 5 was furnished by Dr. F. L. Wellman and is probably the one described as No. 5 in the work of Wellman and Blaisdell.[8] If this supposition is correct, the isolate originally came from Bohn as Missouri isolate No. 7. Missouri Nos. 6 and 10 were reisolated from *Lycopersicon esculentum* × *L. pimpinellifolium* (acc. 160) hybrids inoculated with a mixture of cultures at Columbia, Mo. Single-spored cultures were not used. When mixed cultures of the fungus were used the isolates

[8] WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

were grown separately and mixed in equal proportions at the time of inoculation.

RESULTS

TESTS WITH MIXED CULTURES

Preliminary experiments for comparing the pathogenicity of a mixture of Missouri isolates 6 and 10 with that of a mixture of Washington 5 and Ohio 39 were performed at both experiment stations. The plants tested for resistance included the parental varieties Bonny Best and *Lycopersicon pimpinellifolium* (acc. 160), their F_1, F_2, and F_3 progenies; the backcross to Bonny Best and the F_2 of this backcross; F_2 progenies from crossing *L. pimpinellifolium* (acc. 160) with *L. esculentum* vars. Indiana Baltimore and Early Stone; and F_1 hybrids, Bonny Best × Accession 160, outcrossed to *L. esculentum* vars. Globe and Indiana Baltimore.

Bonny Best was completely susceptible in tests with both mixtures. *L ycopersicon pimpinellifolium* (acc. 160) was resistant to the Missouri mixture but segregated for resistance and susceptibility to the Ohio-Washington mixture. The latter mixture also caused a much higher incidence of disease in the F_1, F_2, and selfed backcross progenies. Six F_3 progenies and 6 selfed backcross progenies of the cross Bonny Best × *L. pimpinellifolium* (acc. 160) were tested for resistance. A total of 221 plants among the 6 F_3 progenies was tested for wilt resistance with the Missouri mixture. Ninety-one plants, or 41.2 percent, were diseased. A total of 214 plants was tested for resistance with the Ohio-Washington mixture. One hundred and thirteen, or 52.8 percent, were diseased. A chi-square test for homogeneity between the 2 groups gave the significant value $\chi^2=5.86$ ($df=1$ $P<5$ percent), thus indicating a difference in pathogenicity between the mixtures. Of the 6 selfed backcross progenies tested, a total of 234 plants was tested for resistance to the Missouri and 230 for resistance to the Ohio-Washington mixture. The former produced 128 diseased plants, or 54.7 percent infection, whereas the latter produced 166 diseased plants or 72.1 percent infection. A chi-square test for homogeneity between the percentage of diseased plants from the 2 groups gave the significant value $\chi^2=15.3$ ($df=1$ $P<1$ percent), again indicating a difference in pathogenicity between the 2 mixtures.

Selfed backcross and F_3 progenies which were homozygous for susceptibility to the Missouri mixture were likewise susceptible to the Ohio-Washington mixture. Progenies which segregated for resistance and susceptibility to the Missouri mixture also segregated for resistance to the Ohio-Washington mixture. However, the number of plants infected by the latter mixture was significantly greater than that infected by the former mixture. For example, among 10 segregating progenies inoculated with the 2 mixtures, the Missouri mixture produced disease in 70 plants among 369, or 19.0 percent infection, whereas the Ohio-Washington mixture produced disease in 157 plants among 347, or 45.2 percent infection. A chi-square test for homogeneity between the two groups gave the significant value $\chi^2=57.0$ ($df=1$ $P<1$ percent), indicating a difference in pathogenicity between the 2 mixtures. Progenies homozygous for resistance to the Missouri mixture segregated for resistance and susceptibility to the Ohio-Washington mixture.

It appeared that at least one of the isolates in the Ohio-Washington mixture possessed a type of pathogenicity which differed from that of the Missouri isolates and that the factor which governed resistance to Missouri isolates was at most only partly effective against one or both of the isolates in the Ohio-Washington mixture.

The results of the preliminary experiments at Ohio and Missouri were in general agreement, although the number of diseased plants was usually greater in Ohio, where the Ohio method of inoculation was used.

TESTS WITH INDIVIDUAL CULTURES

Certain of the parent varieties and F_1 and F_2 hybrid generations (Table 1) were tested for susceptibility to the four isolates separately in Ohio and Missouri. The varieties Bonny Best and Globe were very susceptible in each test. *Lycopersicon pimpinellifolium* (acc. 160) and the F_1 hybrid progeny with Bonny Best proved highly resistant to Missouri isolates 6 and 10 at both locations. With isolate Washington 5 the incidence of disease was higher than with the Missouri isolates in the resistant parent and in the F_1 generation progeny. With the Ohio isolate, the incidence of disease was significantly greater than with the other three isolates.

TABLE 1.—*Pathogenicity of individual cultures of Fusarium oxysporum f. lycopersici*

Pedigree [1]	Location	Cultures Tested															
		Missouri No. 6				Missouri No. 10				Washington No. 5				Ohio No. 39			
		Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
		No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.
		Parental varieties															
Bonny Best	Missouri	0	0	29	100.0	0	0	26	100.0	0	0	32	100.0	0	0	35	100.0
Bonny Best	Ohio	0	1	71	100.0	----	----	----	----	----	----	----	----	0	1	23	100.0
Globe	do	2	1	45	95.8	0	1	23	100.0	1	1	46	97.9	1	0	39	97.5
Acc. 160	Missouri	35	1	0	2.8	38	0	0	0	38	2	0	5.0	20	20	8	58.3
Acc. 160	Ohio	24	0	0	0	24	0	0	0	18	1	5	25.0	4	3	10	76.5
		F_1 Generation															
(BB × Acc. 160)	Missouri	14	0	0	0	13	0	0	0	9	2	0	18.2	3	4	2	66.7
(BB × Acc. 160)	Ohio	9	3	1	30.8	8	5	0	38.5	4	4	5	69.2	2	2	9	84.6
		F_2 Generation															
(BB × Acc. 160)	Missouri	----	----	----	----	36	6	4	21.7	36	3	6	20.0	36	4	8	25.0
(IB × Acc. 160)	do	----	----	----	----	265	22	36	18.0	244	18	59	24.0	202	50	94	41.6
(IB × Acc. 160)	Ohio	14	4	6	41.7	11	3	10	54.2	11	3	8	50.0	1	2	20	95.7
(ES × Acc. 160)	Missouri	----	----	----	----	247	27	42	21.8	208	21	57	27.3	185	48	84	41.6

[1] Abbreviations: BB = Bonny Best; IB = Indiana Baltimore; ES = Early Stone; Acc. 160 = *Lycopersicon pimpinellifolium* (acc. 160).

The reactions of the F_2 progenies were similar, as shown in table 1, with respect to the Missouri and the Washington isolates, but the Ohio isolate again caused a decidedly higher percentage of diseased plants. When the number of healthy plants and the number of diseased plants in the four F_2 progenies resulting from inoculation with the three cultures, Missouri 10, Washington 5, and Ohio 39, were compared separately for homogeneity the chi-square values 0.35, 50.34, 13.72, and 31.23 were obtained. At the 1-percent point with two degrees of freedom, only one progeny (BB × Accession 160) appeared to be homogeneous in its reaction to the three cultures. The other three progenies were significantly heterogeneous in their reactions. These three progenies appeared to react similarly to the cultures Missouri 10 and Washington 5, but differently to Ohio 39. Only one of the four F_2 progenies, when inoculated with the Ohio 39 culture, appeared to segregate for resistance and susceptibility according to a 3:1 ratio. When the number of healthy and diseased plants in the other progenies were compared individually for goodness of fit to a 3:1 ratio, the chi-square values 50.96, 64.22, and 46.81 were obtained, indicating significant deviations. It is not known why the F_2 progeny (BB × Accession 160) reacted similarly to all three cultures, especially since the F_1 and F_3 progenies of the same cross reacted so differently (tables 1 and 2).

TABLE 2.—*Pathogenicity of individual cultures of Fusarium oxysporum* f. *lycopersici to selfed backcross progenies, tested at Wooster, Ohio*

Pedigree[1]	Cultures tested											
	Missouri No. 6				Washington No. 5				Ohio No. 39			
	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
	No.	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*Pct.*
[(BB×Acc. 160)×BB]—17	0	2	22	100.0	0	1	23	100.0	1	1	22	95.8
[(BB×Acc. 160)×BB]—1	1	0	23	95.8	0	0	24	100.0	0	0	16	100.0
[(BB×Acc. 160)×BB]—18	1	2	21	95.8	0	3	21	100.0	0	5	19	100.0
[(BB×Acc. 160)×BB]—20	1	2	18	95.2	0	0	20	100.0	0	2	19	100.0
[(BB×Acc. 160)×BB]—10	2	0	22	91.7	1	0	23	95.8	2	0	14	87.5
[(BB×Acc. 160)×BB]—3	2	0	22	91.7	2	1	21	91.7	1	0	15	93.8
[(BB×Acc. 160)×BB]—11	3	0	21	87.5	2	2	20	91.7	3	0	13	81.3
[(BB×Acc. 160)×BB]—4	3	1	20	87.5	0	1	23	100.0	2	0	14	87.5
[(BB×Acc. 160)×BB]—8	5	1	18	79.2	1	1	22	95.8	1	0	15	93.8
[(BB×Acc. 160)×BB]—6	9	5	10	62.5	14	5	5	41.7	4	2	10	75.0
[(BB×Acc. 160)×BB]—2	13	4	7	45.8	12	2	10	50.0	6	2	8	62.5
[(BB×Acc. 160)×BB]—5	13	3	8	45.8	12	6	6	50.0	0	1	15	100.0
[(BB×Acc. 160)×BB]—13	13	1	10	45.8	13	1	10	45.8	1	1	14	93.8
[(BB×Acc. 160)×BB]—15	13	4	7	45.8	10	4	10	58.3	3	2	19	87.5
[(BB×Acc. 160)×BB]—21	13	5	6	45.8	13	3	7	43.5	1	2	21	95.8
[(BB×Acc. 160)×BB]—14	16	2	6	33.3	19	0	5	20.8	4	0	12	75.0
[(BB×Acc. 160)×BB]—9	17	4	3	29.2	10	1	13	58.3	3	0	13	81.3
[(BB×Acc. 160)×BB]—12	17	2	5	29.2	19	1	4	20.8	4	1	11	75.0
[(BB×Acc. 160)×BB]—16	17	2	5	29.2	16	2	6	33.3	1	3	20	95.8
[(BB×Acc. 160)×BB]—7	19	3	2	20.8	15	4	5	37.5	2	0	14	87.5
Total or average	178	43	256	62.7	159	38	278	66.5	39	22	304	89.3

[1] Abbreviations: BB=Bonny Best; Acc. 160=*Lycopersicon pimpinellifolium* (acc. 160).

The results obtained when the three isolates, Missouri 6, Washington 5, and Ohio 39 were tested for pathogenicity against 20 selfed backcross progenies are shown in table 2. The mean percentages of

diseased plants resulting from inoculation with the Missouri and Washington cultures were similar, 62.7 and 66.5, respectively. The mean percentage of diseased plants caused by the Ohio isolate, 89.3, was decidedly higher. The increased percentage of diseased plants caused by the Ohio culture occurred in the segregating progenies and is particularly noticeable in progenies 5, 7, 9, 12, 13, 14, 15, 16, and 21. These segregated for resistance and susceptibility to the Missouri

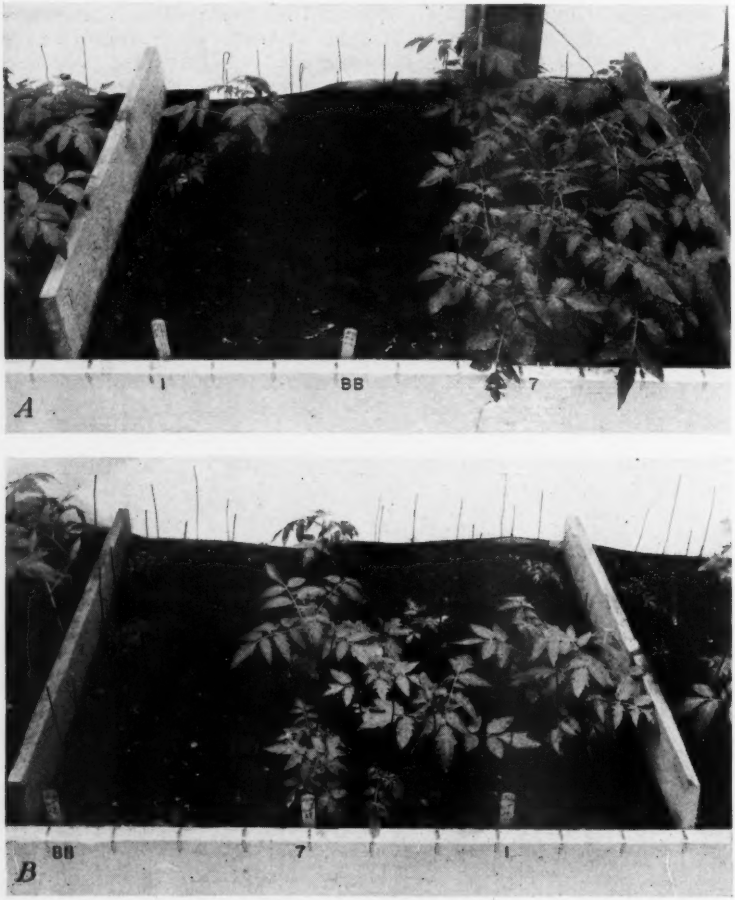

FIGURE 1.—Results of inoculations with Missouri isolate No. 6 (*A*) and Ohio isolate No. 39 (*B*) on Bonny Best, BB, and two selfed backcross progenies No. 1, susceptible, and No. 7, segregating for resistance to the Missouri isolate. Note that Bonny Best was completely susceptible in tests with both isolates.

and Washington isolates but were generally susceptible to the Ohio isolate. Among the progenies segregating for resistance and susceptibility to the Missouri and Washington isolates, the Ohio isolate caused 85.5 percent diseased plants.

Progenies 1, 3, 4, 8, 10, 11, 17, 18, and 20 were apparently homozygous for susceptibility to the Missouri and Washington isolates and were likewise highly susceptible to the Ohio isolate. The percentages of diseased plants caused by Missouri 6, Washington 5, and Ohio 39 were 91.5, 97.3, and 93.9, respectively.

The number of healthy and diseased plants in the 20 selfed backcross progenies inoculated with Ohio isolate 39 did not vary greatly. However, when a chi-square test for homogeneity was made, the significant value $\chi^2=38.27$ ($df=19$ $P<1$ percent) was obtained, indicating that the progenies did not segregate uniformly. The results show that segregation occurred within some of the individual progenies but are inconclusive as to whether the ratio of diseased and healthy plants differed between the progenies. A photograph showing the reaction of two progenies (No. 1, susceptible, and No. 7, segregating) in tests for resistance to two isolates, Missouri No. 6 and Ohio No. 39, is presented in figure 1.

TABLE 3.—*Pathogenicity of individual isolates of Fusarium oxysporum f. lycopersici to advanced generation progenies, tested at Columbia, Mo.*

Progeny [1]	Cultures tested															
	Missouri No. 6				Missouri No. 10				Washington No. 5				Ohio No. 39			
	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased	Healthy	Vascular discoloration	Severely diseased	Total diseased
	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.
	PROGENIES HOMOZYGOUS FOR RESISTANCE TO MISSOURI AND WASHINGTON CULTURES															
10-1085-9	36	0	0	0	46	0	0	0	38	0	0	0	24	12	7	44.2
10-1005-3	40	0	0	0	46	0	0	0	38	1	0	2.6	11	18	12	73.2
10-703-14	45	0	0	0	38	0	0	0	36	0	0	0	10	14	15	74.4
Total or average	121	0	0	0	130	0	0	0	112	1	0	0.9	45	44	34	63.4
	PROGENIES HOMOZYGOUS FOR SUSCEPTIBILITY TO MISSOURI AND WASHINGTON CULTURES															
10-105-7	0	0	42	100.0	0	0	42	100.0	0	1	39	100.0	1	0	43	97.7
10-644-6	1	0	15	93.8	0	0	16	100.0	0	0	19	100.0	1	1	17	94.7
M-140-5-1	0	0	38	100.0	1	0	38	97.4	0	0	44	100.0	1	1	45	97.9
Total or average	1	0	95	99.0	1	0	96	99.0	0	1	102	100.0	3	2	105	97.3

[1] Pedigrees: 10-1085-9. E × Acc.160 × BD × Bis × Bis × Bis, s, × GB, fourth self.
10-1005-3. E × Acc.160 × BD × Bis × Bis × BD, fifth self.
10-703-14. E × Acc.160 × BD, s, × Pond, s, s, × GB, fourth self.
10-105-7. BB × Acc.160 × BB × BB × WB, fourth self.
10-644-6. E × Acc.160 × BD × Bis × Bis, s, s, × EB, fourth self.
M-140-5-1. E × Acc.160 × BD × BB × Pond, fifth self.

Abbreviations: BB=Bonny Best; BD=Break O'Day; Bis=Bison; E=Earliana; EB=Early Baltimore; GB=Greater Baltimore; Acc.160=*Lycopersicon pimpinellifolium* (acc. 160); Pond=Ponderosa; WB=White Beauty; s=selfed.

Table 3 embodies results obtained from inoculation of six advance generation progenies, three homozygous for resistance and three homozygous for susceptibility to the Missouri isolates. The progenies homozygous for susceptibility to the Missouri isolates proved equally

susceptible to the other isolates. The three resistant progenies were highly resistant to the Missouri and Washington isolates but segregated for resistance and susceptibility to the Ohio isolate. This segregation does not appear to be uniform and a chi-square test for homogeneity gave the significant value $\chi^2=10.57$ ($df=2$ $P<1$ percent), which suggests that the progenies are different genotypes. The difference appears to be due to the progeny 10–1085–9. The other two progenies appear to be homogeneous.

Ten advanced-generation progenies, 5 homozygous and 5 heterozygous for resistance to the Missouri isolates, selected on the basis of their behavior in glasshouse and field tests during the previous season, were compared for resistance to isolates Missouri 10 and Ohio 39. The plants inoculated with the Ohio isolate were grown to maturity. The results, presented in table 4, show that of the 5 progenies homozygous for resistance to the Missouri isolate 3 segregated for resistance and susceptibility to the Ohio isolate and 2 were completely susceptible. Four of the progenies heterozygous for resistance to the Missouri cultures were also heterozygous for resistance to the Ohio culture, and 1 was completely susceptible.

The pathogenicity of Ohio isolate 39 appears to differ qualitatively from that of the other isolates. When susceptible commercial varieties or hybrid progenies homozygous for susceptibility to the Missouri isolates were inoculated with Ohio 39 the resultant disease symptoms did not develop earlier or with greater severity than when these progenies were inoculated with the Missouri isolates. Indeed, the Ohio isolate would have been regarded as slightly less virulent than the Missouri and Washington isolates had the inoculations been confined to such genotypes. It was only when progenies with the factor for resistance to the Missouri and Washington isolates obtained from *Lycopersicon pimpinellifolium* (acc. 160) were inoculated that the unusual pathogenicity of the Ohio isolate became evident.

Other commercial varieties tested proved susceptible to Ohio 39. For example, in a test terminated 7 weeks from planting, Break O'Day, Rutgers, and Pan America became diseased to the extent of 86, 81, and 71 percent, respectively. The variety Pan America, described by Porte and Walker [9] in 1941, was developed from the cross *Lycopersicon pimpinellifolium*, P. I. 79532, (possibly identical with Bohn and Tucker's Accession 160) × Marglobe, with backcrosses to Marglobe. The variety proved highly resistant to the Missouri isolates but only slightly resistant to Ohio 39.

The four isolates, Missouri 6 and 10, Washington 5, and Ohio 39, were compared in culture on potato-dextrose, maize-meal, and oatmeal agars. They proved similar in growth rates, in temperature-growth relations, and in the morphology of micro- and macrocinidia. The isolates varied in certain growth characters. Some produced abundant aerial mycelium and caused little coloration in the medium. With others the mycelium was appressed to the surface of the agar, which assumed various shades of lavender or purple. Ohio 39 produced saltant sectors in potato-dextrose-agar cultures. Transfers from the sectors yielded lines with various growth types. The behavior of the

[9] PORTE, W. S., and WALKER, H. B. THE PAN AMERICA TOMATO, A NEW RED VARIETY HIGHLY RESISTANT TO FUSARIUM WILT. U. S. Dept. Agr. Cir. 611, 6 pp., illus. 1941.

four isolates in culture was in accord with the results reported by Wellman and Blaisdell [10] in a study of a collection of isolates of the wilt fungus. From these observations the four isolates appear to be *Fusarium oxysporum* f. *lycopersici*.

TABLE 4.—*Pathogenicity of Missouri isolate No. 10 and Ohio isolate No. 39 to advanced generation progenies in tests at Columbia, Mo.*

[Seeds planted Mar. 7, 1942; plants transplanted and inoculated Mar. 28 and again May 15. Plants inoculated with No. 10 removed May 15; plants inoculated with Ohio 39 grown to Nov. 11, 1942]

Progeny [1]	Cultures tested														
	Missouri No. 10					Ohio No. 39									
	Wilt to May 15					First inoculation Mar. 28, 1942			Plants lost transplanting	Second inoculation May 15, 1942, records taken Nov. 11, 1942					Total diseased both transplantings
	Plants	Healthy	Vascular discoloration	Seriously diseased	Total diseased	Plants	Diseased	Diseased		Transplanted	Healthy	Vascular discoloration	Seriously diseased	Total diseased	
	No.	No.	No.	No.	Pct.	No.	No.	Pct.	No.	No.	No.	No.	No.	Pct.	Pct.
	LINES HOMOZYGOUS FOR RESISTANCE TO MISSOURI ISOLATES														
10–25–1	79	79	0	0	0	78	46	59.0	2	30	7	7	16	76.7	90.8
10–26–3	79	77	1	1	2.5	80	69	86.3	3	8	0	0	8	100.0	100.0
10–31–4	80	80	0	0	0	80	69	86.3	2	9	0	1	8	100.0	100.0
10–32–1	75	75	0	0	0	79	43	54.4	3	33	12	10	11	63.6	84.2
10–38–1	80	80	0	0	0	79	24	30.4	2	53	27	11	15	49.1	64.9
Total or average	393	391	1	1	.5	396	251	63.4	12	133	46	29	58	65.4	88.0
	LINES HETEROZYGOUS FOR RESISTANCE TO MISSOURI ISOLATES														
10–6–8	85	76	4	5	10.6	78	63	80.8	3	12	3	4	5	75.0	96.0
10–33–12	80	66	6	8	17.5	80	48	60.0	0	32	18	11	3	43.7	77.5
10–41–5	76	71	1	4	6.6	81	63	77.8	5	13	6	2	5	53.8	92.1
10–43–4	78	74	1	3	5.1	80	62	77.5	5	13	3	0	10	76.9	96.0
10–70–4	78	75	2	1	3.8	81	73	90.1	6	2	0	1	1	100.0	100.0
Total or average	397	362	14	21	8.8	400	309	77.2	19	72	30	18	24	58.3	92.1

[1] Pedigrees: 10–25–1, 10–26–3, 10–31–4, 10–32–1, 10–38–1. BB × Acc. 160 × BB × BB × BD, third self.
10–6–8. BB × Acc. 160 × Ox × Bis × BD, third self.
10–33–12. BB × Acc. 160 × BB × BB × BD, third self.
10–41–5, 10–43–4. BB × Acc. 160 × BB × BB × BD, s, × ES, first self.
10–70–4. BB × BB × (BB × Acc. 160), s, × ES, first self.
Abbreviations: BB=Bonny Best; BD=Break O'Day; Bis=Bison; ES=Early Stone; Acc. 160=*Lycopersicon pimpinellifolium* (acc. 160); Ox=Oxheart; s=selfed.

DISCUSSION

The Missouri and Washington isolates exhibited the same type of pathogenicity, although the latter consistently caused a few more diseased plants. On the other hand, the Ohio isolate appeared to possess an entirely different type of pathogenicity. It proved pathogenic to *Lycopersicon pimpinellifolium* (acc. 160) and caused signifi-

[10] WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

cantly higher percentages of diseased plants in the F_1, F_2, and backcross progenies, and in advanced generations selected for resistance to the Missouri isolates. Resistance to the Missouri and Washington isolates appeared to segregate according to a simple Mendelian ratio, when tested at Missouri with the Missouri technique; the inheritance of resistance to the Ohio isolate appeared to be more complicated, regardless of where the tests were made, as shown by the high percentage of diseased plants. The Ohio isolate caused much larger percentages of infected plants in some of the selfed backcross progenies than did the Missouri or Washington isolates (table 2). The differences in susceptibility indicated that tomato lines highly resistant to the Missouri and Washington isolates might be very susceptible to the Ohio isolate. Some of the progenies apparently homozygous for resistance to the Missouri isolates, proved highly susceptible to the Ohio isolate (table 4).

Since all progenies homozygous for susceptibility to the Missouri and Washington isolates were likewise susceptible to the Ohio isolate, it appears that the absence of the factor for resistance to the former results in susceptibility to the Ohio isolate. Some progenies homozygous for resistance to the Missouri and Washington isolates were completely susceptible to the Ohio isolate, whereas others segregated for resistance and susceptibility. The results suggest that the factor for resistance to the Missouri and Washington isolates must be present if there is to be resistance to the Ohio isolate, but that this factor alone is not sufficient to assure resistance to it. The presence of an undetermined number of complementary resistance factors, in addition to the factor for resistance to the Missouri and Washington isolates, is probably necessary for resistance to the Ohio isolate. If this is true it is to be expected that tomato lines homozygous for resistance to the Missouri and Washington isolates should exhibit various degrees of resistance, or complete susceptibility to the Ohio isolate, depending on the presence and the number of complementary factors in the genotype.

The advanced generations tested for resistance (tables 3 and 4) were developed at the Missouri station and were obtained by backcrossing to the susceptible parent, outcrossing to commercial varieties, several generations of selfing, and with selection always directed toward resistance to the Missouri isolates. If complementary resistance factors are necessary and enter the genotype from the *Lycopersicon pimpinellifolium* parent, it is not surprising that some or all of them were lost, resulting in lines homozygous for resistance to the Missouri isolates but heterozygous for resistance or homozygous for susceptibility to the Ohio isolate.

The demonstration of physiologic races of the fungus complicates the problem of development of varieties of tomatoes homozygous for resistance to all forms of the fusarium wilt organism. However, it does not appear to preclude the possibility of developing varieties that are highly resistant to all forms. This is brought out particularly in table 4, where it is shown that some resistance to Ohio 39 is retained even after several backcrosses to susceptible varieties without selection for resistance to it.

Little is known regarding the prevalence or distribution of physiologic races of *Fusarium oxysporum* f. *lycopersici*. Such evidence as

is available indicates that they are not common. Bohn and Tucker,[11] in tests with 39 isolates, and Wellman and Blaisdell,[12] in tests with 29 isolates, found *Lycopersicon pimpinellifolium* to be very resistant to all. So far as the writers are aware, Pan America has proved consistently resistant in various areas. Selections from hybrids developed at Missouri, resistant to the Missouri isolates, proved resistant when grown in infested fields in Indiana, Alabama, Louisiana, Texas, California, Kansas, and South Africa.

The method of inoculation used at Ohio gave a higher percent of diseased plants than the method used at Missouri. However, this difference can probably be accounted for by the shock and root injury produced by the former method. Regardless of the fact that the incidence of disease was higher in the Ohio tests, the results secured at both locations appear to show that the Ohio isolate has a different type of pathogenicity from that of the Missouri and Washington isolates.

SUMMARY

A race of *Fusarium oxysporum* f. *lycopersici* has been isolated in Ohio which appears to have a distinctive type of pathogenicity.

A comparison of the pathogenicity of isolates from various sources revealed that tomato lines resistant to isolates of the types previously studied may be completely susceptible to the Ohio isolate.

The inheritance of resistance to the Ohio race appears to involve the gene for resistance to the Missouri race and an undetermined number of complementary genes.

Lycopersicon pimpinellifolium, accession 160, appears to be segregating for resistance and susceptibility to the Ohio race, possibly due to heterozygosity for the complementary resistance factor or factors.

All commercial varieties of tomato tested were susceptible to the Ohio isolate.

[11] BOHN, G. W., and TUCKER, C. M. See footnote 4.
[12] WELLMAN, F. L., and BLAISDELL, D. J. See footnote 6.

U. S. GOVERNMENT PRINTING OFFICE: 1945